CONCERNING THE ORGANIZATION OF THE SPORE MOTHER-CELLS OF MARSILIA QUADRIFOLIA

BY

WILLIAM GEORGE MARQUETTE

A THESIS SUBMITTED FOR THE DEGREE OF DOCTOR OF PHILOSOPHY
UNIVERSITY OF WISCONSIN
1907

[REPRINTED FROM THE TRANSACTIONS OF THE WISCONSIN ACADEMY OF SCIENCES, ARTS AND LETTERS, VOLUME XVI, PART I, PAGES 81-106]

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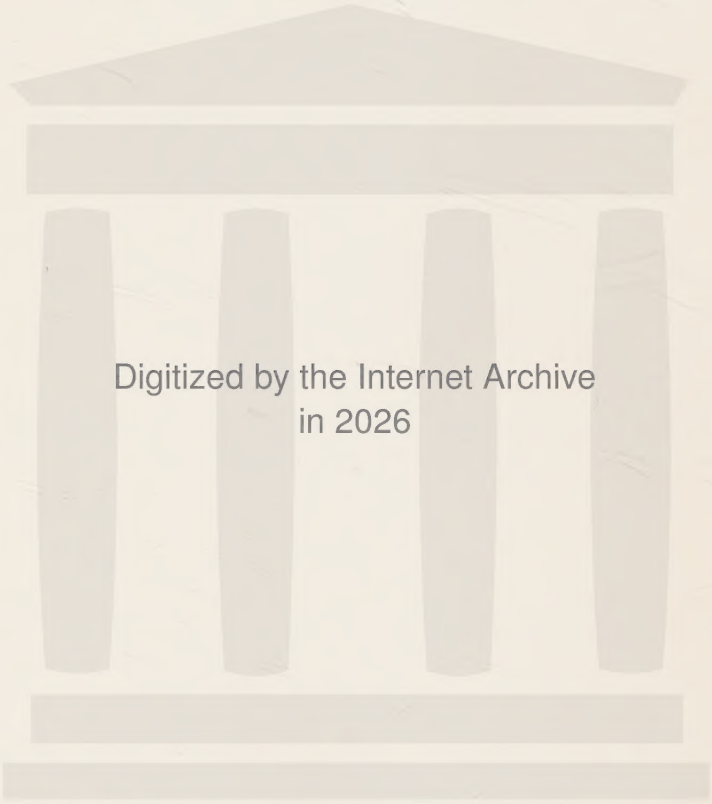
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CONCERNING THE ORGANIZATION OF THE SPORE MOTHER-CELLS OF MARSILIA QUADRIFOLIA.

W. MARQUETTE.

(With Plates VIII and IX.)

In a recent paper,[1] I incidentally called attention to the fact that during the synapsis stages of spore mother-cells of *Marsilia quadrifolia* the starch grains, which are almost invariably present at that time, are aggregated in a definite region of the cell. In connection with the translocations which the starch grains in young leaf-cells of *Isoetes* undergo during mitosis, this raised the question as to the behavior of the starch aggregation in the spore mother-cells of *Marsilia* during mitosis, and as to whether there is a permanent polar organization of the cells here similar to that found in the vegetative cells of *Isoetes*. Further interest is given to a study of the organization of the cells in the sporocarp of *Marsilia* because of Belajeff's statements[2] regarding the behavior of the blepharoplasts in the microgametophytes of *Marsilia*. Belajeff traces the blepharoplast back several cell-generations from the antherozoid and describes it as behaving during mitosis in all respects like the central body of animal cells, and concludes that they are similar in function.

It is to be expected that a study of the method of spindle formation and cell division in the various cells of the sporo-

[1] Manifestations of polarity in plant cells which apparently are without centrosomes. *Beih. z. Bot. Centralbl.*, xxi, Abt. i:297. 1907.

[2] Belajeff, W.: Ueber die Centrosome in den spermatogenen Zellen. *Ber. d. deut. bot. Ges.*, xvii:199. 1899.

carp will throw light on the question as to the presence in the sporocarp cells of the blepharoplast, or of structures related to it and finally giving rise to it. It was with these problems especially in view that I undertook the investigations here described.

The material was obtained from plants of *M. quadrifolia* growing in the greenhouses connected with the botanical laboratories of the University. Flemming's stronger and Flemming's weaker chrom-osmium-acetic fixing fluids, as well as some modifications of the same, were used for the most part and gave the best results. In general, the original formulae as published by Flemming gave at least as good if not better results than the various modifications of the same which have from time to time been recommended. Several sublimate mixtures were also used but did not give results equal to those of the chrom-osium-acetic mixtures, although they gave valuable control preparations. For staining, Flemming's triple stain and Heidenhain's iron-haematoxylin were used almost exclusively. The sporocarps of *Marsilia* offer a most favorable material for cytological study. They are readily obtained in all stages of development, fix well, and, contrary to what might be expected, section easily. The different parts of the cell take a good differential stain with the triple stain, so that preparations of unusual brilliancy result.

Furthermore, the cells found in the sporocarp offer an interesting variety. There are the ordinary vegetative or somatic cells of the sporocarp walls, etc., which may be looked upon as typical vegetative cells. In young sporocarps these are actively dividing, so that abundant instances of the various stages of spindle formation are to be found. The tapetal cells represent another type of somatic cells, i. e. cells which are soon to disintegrate. For such cells, aberrant nuclear and cell divisions have been described for a large number of plants and animals. Then there are the "germ cells," the spore mother-cells and their ancestors. In the spore mother-cells we have cells which have severed their structural connection with other cells, so that they do not form an architectural part of a tissue, at least not in the later stages of their development when they are begin-

ning to divide. A tissue cell is subject to specific and different environmental conditions on its different sides, certain factors of which remain constant for at least considerable periods. With the free spore mother-cells it is otherwise. While the conditions are not the same in all directions in the spore sac, any part of a free spore mother-cell apparently is as likely to lie in a given position as any other; furthermore, there is some evidence that the spore mother-cell rotates more or less previous to and during division, so that, whatever differences there may be in the environment of a spore mother-cell, there are no fixed and specific differences with respect to the environment between different parts of the cell, as is the case with cells forming part of a tissue complex, and in this difference a possibility is provided for a difference in the method of formation and orientation of the karyokinetic figure in the two cases.

In the following, I shall take up only the organization of the spore mother-cells and the processes of division occurring in them. I have concerned myself especially with the activities of the cytoplasm. The points in Strasburger's paper,[8] which appeared when my work was practically completed, bearing on the problems taken up here will be considered as they come up.

Shortly after their formation, the sixteen spore mother-cells of a spore sac each show an approximately centrally placed nucleus in a dense cytoplasm. The more solid constituents of the cytoplasm are closely packed and usually show no inclusions at this time. Nucleus and cell are of relatively small size as compared with later stages. A period of growth now sets in during which the size of the nucleus and cell increase greatly up to the time of synapsis. During this period of growth, specific and characteristic changes occur in the cytoplasm. At what is apparently a variable period in the growth of the spore mother-cell, starch grains make their appearance in the cell. Just before they appear, the cytoplasmic strands, or lamellae, of the spore mother-cell are relatively coarse and of irregular outline. For the most part, these

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8-Strasburger, E.: Apogamie bei *Marsilia*. *Flora*, xcvii:123. 1907.

strands tend to take the orange of the triple stain, but here and there are portions staining blue. In some cases, larger, blue-staining granules are found at intervals along the strands. On the outer side of the cell, in the position finally to be occupied by the starch mass, a relatively large, elongated, clear region is frequently to be found. At times it is of regular outline, lying about midway between nucleus and outer cell wall, its short diameter equal to about one-quarter the distance between the nucleus and cell wall. At other times this region is more irregular in outline and may branch somewhat. In any case, it is bounded by comparatively heavy, blue-staining elements of the cytoplasm which frequently show larger granules lying close against their inner side; the upper left corner of Figure 2 shows part of such a region. The appearance of this region in the cytoplasm suggests the polar structures of *Isoetes,* especially as they are found in the early prophases and practically free from starch. Unfortunately, I am at present unable to say anything further concerning the significance or subsequent history of this appearance in the cytoplasm of the spore mother-cells of *Marsilia.* The nucleus at this time shows the chromatin finely divided and aggregated in clusters of varying shapes; frequently long, irregular threads of chromatin run out from these clusters. Numerous nucleoles are present.

When the starch grains first appear, they are usually more or less scattered in the cell and rather widely separated from each other; frequently it seems they tend to lie near the nucleus. Soon, however, this scattered arrangement gives place to a more definite one; the starch grains are found at a specific region of the cell, being located almost invariably between the nucleus and the wall of the cell facing towards the outside of the spore sac. Frequently the starch grains lie in a quite distinctly bounded group, often of a cylindrical shape, one end in contact with the nucleus and the other reaching almost to the cell wall (Fig. 3). While most of the starch grains at this stage lie in this region, it is to be noted that usually some of them lie outside of it. In the cell represented in Figure 3, in a higher plane than that at which the

drawing is made, a number of starch grains, arranged in a single row, extend fully halfway around the nucleus.

The starch grains at this time stain a deep blue, and are not as closely packed as at a somewhat later stage. The more or less cylindrical region lying between nucleus and outer cell wall contains, in addition to the starch grains, strands with a granular structure which run here and there between the starch grains, probably forming more or less of a network. Besides these strands, there seems to be a diffuse, finely granular, blue-staining substance scattered throughout this region. It is difficult to make out with certainty the structure of the material between the starch grains, because the latter are numerous and stain heavily, so that at any particular focus considerable light is cut off by the overlying and underlying portions; but it seems certain that there is a finely divided substance staining blue in the triple stain between the starch grains. The abrupt ending of this material outside of the starch grains seems to be at least to a considerable extent responsible for the fairly sharp delineation of this region against the remainder of the cytoplasm. The cytoplasm as a whole is still rich in its more solid constituents, although the proportion of vacuolar space has become somewhat larger than it was in the earlier stages. The strands have become more drawn out, look more like fibers, and many of them show perhaps a greater affinity for the blue stain. Also conspicuous at this time are unusually long strands, which frequently lie in groups, converging at one end and spreading apart at the other. In addition, there are dense-staining granules scattered here and there in the cytoplasm.

The cell apparently has reached its full size, although the nucleus, which at this stage lies near the inner cell-wall, seems as yet noticeably smaller than at the time of synapsis. The chromatin is in the form of strands of fairly regular outline and uniform diameter, which loop and coil around considerably, often traversing the nuclear cavity. Free ends of such strands are not apparent, although if they lay at the points where the strands come in contact with the nuclear membrane they could easily be overlooked.

The starch masses of the sixteen spore mother-cells in a spore sac are placed so as to be removed as far as possible from each other. This suggests that the position of the starch mass in the cell may be determined by metabolic requirements. The region of the starch mass is probably at times the seat of great metabolic activities in the cell. At least the greater part of interchange of materials between the spore mother-cells and the exterior occurs through the tapetum; on the other hand, there is considerable evidence that the nucleus is intimately concerned with the metabolic activities of the cell.[4] Now, the location of the starch masses of the sixteen spore mother-cells of a spore sac is such that they lie in a position of maximum advantage with reference to an interchange of metabolic materials and products with the surrounding tapetum on the one hand and to proximity to the nucleus on the other.

It is to be noted that the spore mother-cell at this stage presents a distinct polar organization. A line drawn through the center of the starch aggregation and through the center of the nucleus would represent the main axis of the cell. The first conspicuous evidence of this organization is the appearance of the starch mass at a definite place in the cytoplasm. It is to be noted further that the nucleus itself as yet shows no evidence of polarity. In synapsis, however, which soon sets in, the nucleus presents several most interesting features bearing on its organization at this time. The synaptic mass, as is commonly the case, collects on one side of the nucleus, and, as I previously noted, in *Marsilia* it is invariably on the side of the nucleus next to the starch aggregation in the cytoplasm. This stage is of long duration, so that great numbers of cells are found in this condi-

[4] Küster (Ueber die Beziehungen der Lage des Zellkerns zu Zellenwachstum und Membranbildung. *Flora*, xcvii:1, 1907) has recently thrown doubt on Haberlandt's work (Beziehungen zwischen Function und Lage des Zellkerns bei den Pflanzen, Jena, 1887) concerning the relation between the nucleus and the metabolic activities of the cell. There are, however, numerous other data pointing to such a relation. I have in mind especially the results obtained on secreting cells, particularly animal cells.

tion.[5] Usually the chromatin strands can be quite readily followed throughout this stage, but at times unusually dense synaptic masses are met with in which the individual chromatin strands are practically indistinguishable. A striking feature of synapsis in *Marsilia* is that a large number of the strands converge toward a point on the nuclear membrane which lies on, or close to, the axis of the cell. Furthermore, at this point, closely in contact with the nuclear membrane, lies a nucleole, sometimes apparently two, differing from the other nucleoles of the nucleus. This comes out especially well in preparations stained with the triple stain. Usually a considerable number of the nucleoles are still present at this stage, and the one lying at the focal point of the chromatin strands almost invariably shows a bright, deep red color, standing out sharply from the blue chromatin strands. The other nucleoles lying here and there in the nuclear cavity, on the other hand, show a somewhat pale grayish-blue color. These figures are frequently of remarkable regularity, standing out with almost diagrammatic clearness.

The chromatin strands during synapsis frequently show more or less of a characteristic orientation. In animal cells, the strands are centered upon the central body and the synaptic mass lies against the nuclear membrane on the side next to the central body.[6] The same is true of the Ascomycete *Phyllactinia.*[7] In the higher plants, the synaptic mass usually lies close to one side of the nuclear membrane, but in general the

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[5] As this was ready to go to press, my attention was called to a paper by Schaffner (Synapsis and synizesis. *Ohio Naturalist*, vii:41, 1907), in which he describes and figures synapsis in a sporocarp of *M. quadrifolia* and points out that the synaptic mass lies on the side of the nucleus nearest the sporocarp wall, attributing this orientation, however, to the action of the fixing reagents. There is no occasion to enter into a discussion of this and other similar conclusions of the author concerning synapsis in general.

[6] Farmer, J. B., and Moore, J. E. S.: The maiotic phase in animals and plants. *Quart. Jour. Microscop. Sci.*, xlviii, Pl. XL, Fig. 72. Schreiner, A. und K. E.: Ueber die Entwickelung der männlichen Geschlechtszellen von *Myxine glutinosa. Arch. de Biol.*, xxi, Figs. 52, 71, 170, etc.

[7] Harper, R. A.: Sexual reproduction and the organization of the nucleus in certain mildews. *Carnegie Inst. Publ.* No. 37 (1905), Pl. IV, Figs. 43–45.

figures which have so far been given of synaptic stages show no conspicuous centering of the chromatin strands upon any particular region, although occasionally a nucleus has been figured showing a rather conspicuous orientation of the strands upon a certain part of the nuclear membrane; for example, by Overton for *Helleborus foetidus*[8] (in this case the nucleole is included in the synaptic mass but does not lie in contact with the nuclear membrane), and by Berghs for *Narthecium ossifragum*.[9] In the latter case the strands are centered upon a nucleole lying at the periphery of the nucleus. But in other figures of the same plants given by these authors the nucleoles lie outside of the synaptic mass and the chromatin strands show a less regular course. In *Lilium*, which has been so extensively studied,[10] there seems to be little indication of an orientation of the strands upon any one region, nor is there any regularity in the position of the nucleole. In *Marsilia quadrifolia* the orientation of the chromatin strands during synapsis is apparently as definite and characteristic as in *Phyllactinia* or in any of the animal cells.

The nuclear membrane also shows an interesting dissimilarity between its "polar" and "antipolar" sides. This difference stands out especially well in preparations which are stained rather faintly. The nuclear membrane appears much heavier on the side of the nucleus turned away from the starch mass than on the side next to it. In some cases it almost looks as if a close-fitting cap extending over one-third to two-thirds the surface of the nucleus were symmetrically placed opposite the synaptic mass over the otherwise thin nuclear membrane. If the preparation has a slight excess of the orange stain, this part continues to stain blue and stands out in contrast to the otherwise largely yellow preparation. Whether there actually is a thickening of the nuclear membrane here is perhaps a question. In a normally stained preparation it is seen

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[8] Overton, J. B.: Histologische Beiträge zur Vererbungsfrage. *Jahrb. f. wiss. Bot.*, xlii (1906), Pl. VII, Fig. 46.

[9] Berghs, J.: La formation des chromosomes heterotypiques dans la sporogénèse végétale. *La Cellule*, xxii, Fig. 17.

[10] See the figures of Allen, C. E.: *Jahrb. f. wiss. Bot.*, xlii (1906), Pl. II, and *Ann. Bot.*, xix, Pl. VI.

that numerous deeply-stained blue granules lie close against the inner side of this part of the nuclear membrane (Fig. 4). They are rarely if ever more than one layer deep, and, although small, stain intensely, so that they are readily made out in a tangential section of the nucleus. These granules are found only under the apparently thicker parts of the nuclear membrane, and undoubtedly are partly responsible for the heavier appearance of this part. As will presently be seen, the arrangement of the cytoplasmic fibers outside of the nucleus is also such as to make this part of the nuclear membrane appear thicker. Blue-staining fibers have become conspicuous in the cytoplasm and form an intertwining network of relatively long fibers, lying for the greater part close to the nucleus. The fibers have a distinctly different appearance now from that presented by the fibrous constituents of the cytoplasm in the earlier stages of the development of the spore mother-cells. They have become smoother, take a brighter blue stain, and stand out in greater contrast to the other constituents of the cytoplasm; here and there they show thickenings and granules lying against them. In a tangential section taking in just a small piece of the surface of the nucleus, these fibers can be seen in great numbers forming a loose tangle about the nuclear membrane. A median section of the cell, however, taken in such a plane that it includes the nucleole at the focus of the chromatin strands, in other words in a plane including the main axis of the cell, shows that the cytoplasmic fibers are not uniformly distributed about the nucleus (Fig. 4). At the side opposite the starch mass they lie closely packed against the nuclear membrane, so closely that frequently it is difficult to make out the individual fibers; the appearance at times suggests that the cytoplasmic fibers are separating, "splitting off" from this denser part of the nuclear membrane. As they approach the region of the starch mass, the fibers separate more from each other and extend farther from the nucleus. When they reach a plane drawn a little above the nucleole at the focal point of the chromatin strands and perpendicular to the main axis of the cell, the fibers frequently bend rather sharply in towards the edges of the starch mass, at times giving rise to a grouping

of the fibers which suggests the beginning of the formation of spindle poles. It is to be noted that if the completed spindle should lie in this plane, its axis would be at right angles to the original "main axis" of the cell. When the fibers reach the starch mass it is practically impossible to trace them further. Except for the blue-staining fibers, the cytoplasm shows a somewhat looser structure than in the preceding stages.

The close packing of the fibers against the nuclear membrane on the side opposite the starch mass also contributes to make this part of the nuclear membrane appear thicker. However, a close study makes it seem probable that the appearance is due to something more than the mere deposition of granular material on the inside of this part of the membrane and the close apposition of cytoplasmic fibers on its outside; this is indicated, among other things, by the frequently abrupt ending of this region. At any rate, the accumulation of this dense, blue-staining material at a specific part of the nuclear membrane requires further study. The remainder of the membrane is exceedingly delicate, often it is scarcely to be made out. It is to be noted that all this development of the conspicuous fibrous system just described has occurred before and at the time of synapsis.

I have called this stage *synapsis*, although there are certain stages passed through by the nucleus at a much earlier period than this which further study may prove to be of importance in connection with the processes usually associated with synapsis. Early in the growth of the spore mother-cell, when the nucleus is still small, a stage is passed through in which the chromatin is aggregated in a dense mass lying at the center of the nucleus (Fig. 1). The chromatin is at this time in the form of finely divided particles and tends to collect into small, denser groups within the larger mass; often strands of varying lengths are formed, and usually one or several loops of chromatin extend out from the central mass into the surrounding clear nuclear cavity. The whole appearance strongly resembles synapsis. It differs from synapsis as usually found in that in this case the contracted chromatin mass lies at the center of the nucleus instead of at the periphery, and in that it occurs at

a much earlier stage in the growth of the cell than is commonly the case.[11] Speaking in terms of current theories, the possibility suggests itself that at this stage the pairing of the homologous chromatin elements may take place, while the subsequent contraction and specific orientation of the chromatin strands is associated with the mechanics of spindle-formation, i. e., with the relation between spindle-fibers and chromatin; and that in this latter conception may lie the explanation of the specific orientation of synaptic masses in general. This specific orientation is as conspicuous as the contraction of the chromatin at this period and is quite unaccounted for on the basis of the theories which find in synapsis a provision for the pairing or fusing of homologous chromatin elements. However, further knowledge of the behavior of the chromatin at this time is needed as a basis for such suggestions. The possibility is not excluded that the early contraction in the spore mother-cells of *Marsilia* may be artifact, although it is too regular in its occurrence to make such a supposition probable. For the present, I shall continue to speak of the later aggregation as synapsis; but it is to be noted that this is preceded by a "spirem" stage!

As the chromatin passes out of synapsis and becomes more uniformly distributed through the nuclear cavity, the fibrous constituents of the cytoplasm become even more conspicuous and usually show a tendency to be arranged in cone-shaped groups of which there are frequently two in a cell. At times a well formed bipolar spindle is developed, the spindle usually lying somewhat tangential to the nucleus (Figs. 5 and 6). Usually this "spindle" lies on the side of the nucleus adjacent to the starch mass. Strasburger[12] figures a similar stage for *M. elata*, and there the same relations seem to obtain between the position of the nucleus, the spindle, and a dense region of

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[11] Generally in the higher plants the nucleus has reached about half or more of its definitive size (linear) by the time of synapsis, although there are exceptions, as in *Galtonia* according to Miyake [*Jahrb. f. wiss. Bot.*, xlii (1906), Pl. III, Figs. 6–12]. In the case of the primary oocyte in animal cells, there is of course usually a tremendous increase in the size of the nucleus after synapsis.

[12] Apogamie bei *Marsilia*. *Flora*, xcvii, Pl. VI, Figs. 71 and 72.

the cytoplasm. Strasburger does not specifically describe this as a starch-containing region, but judging from the similarity between the appearance of this region and that of what is undoubtedly the starch-bearing region of *M. quadrifolia*[13] described by him as "eine einseitige Verdichtung im Zytoplasma", it is probable that it is the same in *M. elata.* Occasionally abundant polar radiations extend from the poles to the cytoplasm on the side of the spindle away from the nucleus, and there may be a pronounced crossing of these radiations in the equatorial region. This crossing necessarily occurs near the surface of the starch mass (the fibers apparently in general do not extend into the region occupied by the starch grains), and is especially conspicuous when the section is such that the greater part of the starch mass is cut away, or when the starch is but faintly stained. In the cell represented in Figure 5, the greater part of the starch lies beneath the plane of the drawing. The poles of the spindle, or, perhaps more accurately, the apices of the cones of fibers, usually do not lie in contact with the plasma membrane, but a short distance inside of it. Occasionally dense aggregations appear in the cytoplasm about the poles, at times also a deeply staining granule is found there; however, there seems to be nothing constant in these formations, which apparently occur just as frequently elsewhere in the cell as at the apices of the fibrous cones. The left hand cell in Figure 6 shows at the upper side such a granule which closely simulates a central body with a hyaline zone and a dense aggregation of cytoplasm at its periphery, such as is common in animal cells. In the preparation, this is, if anything, even more striking than in the drawing. As the figure shows, however, this formation in this case seems to be quite independent of the two groups of spindle fibers which have developed, a fact which alone takes it out of the category of the central bodies as they are known in the lower plant and animal cells—unless, indeed, these groups of fibers should not be the beginning of the spindle figure, a possibility for which, as will be seen later, there is some evidence.

[13] Ibid., Figs. 60 and 61.

By going over a large number of preparations of spore mother-cells, it is possible to pick out a series in which a granule can be shown at all of the various positions which a central body is expected to occupy during the processes of nuclear and cell division, from the stage of the single granule lying close to the membrane of the resting nucleus, and that of its division into two, to the appearance of the single granule lying at the polar side of the reconstructed daughter nucleus. But for every preparation of any stage which shows the formations in question in the expected position, there are dozens in which it either does not appear at all or in which similar formations occur elsewhere in the cell and in varying numbers. There is no occasion to dogmatize concerning these granules and aggregations; it is possible that more complete data will show that they run through a regular cycle and that they play a part in the mechanics of the cell; but as far as our data go at present, there is little or nothing to make this probable. The formation of two cone-shaped groups of fibers at this stage is not invariable. Sometimes a larger number is formed; Figure 5 shows indications of a third cone, and sometimes such sharply defined poles as are shown in Figures 5 and 6 do not seem to be formed at all. While it is common to find the fibers of the cytoplasm collected into two cone-shaped groups at this time, it would appear that they do not directly form the spindle figure. These large cone-shaped groups of fibers are most frequently met with at the time when the chromatin strands are long and thin and show no marked signs of segmentation. Somewhat later, when the thread has thickened considerably and the individual chromosomes are easily made out, the fibers are often in numerous smaller cone-shaped groups, each consisting of but a few fibers. These groups are rarely if ever placed symmetrically about the nucleus, as is the case in the radial stage[14] described for the spore mother-cells of various plants. Instead, the fibers lie more or less tangential to the nucleus on one side, at times suggesting a "spindle wall" as

[14] Osterhout, W. J. V.: Ueber Entstehung der karyokinetischen Spindel bei *Equisetum*. *Jahrb. f. wiss. Bot.*, xxx:159. 1897. Allen, C. E.: The early stages of spindle formation in the pollen mother-cells of *Larix*. *Ann. Bot.*, xvii : 281. 1903.

described by Osterhout for *Agave*,[15] while on the other side of the nucleus the fibers are in small, radially placed, cone-shaped groups whose bases are directed toward the nuclear membrane (Fig. 7). The cell from which Figure 7 is taken shows a number of these groups in a lower plane of the section. As in the preceding stages, the blue-staining fibers stand out in sharp contrast to the other, orange-staining, constituents of the cytoplasm. The starch mass continues to be a conspicuous feature of the cell; in the cell represented in Figure 7, most of it lies above the plane of the drawing.

As the chromosomes contract further and the nucleus goes into a well-marked diakinesis stage, the cone-shaped groups in the cytoplasm are even less in evidence. The fibers are most closely packed in a region about halfway between the nuclear and plasma membranes: from this denser region they extend in to the nuclear membrane, their outer ends converging in small groups here and there (Fig. 8), but there is little suggestion of the arrangement as found in the earlier "bipolar" stages. The fibers are fairly uniformly distributed about the nucleus excepting in the region occupied by the starch mass. Here they are much fewer in number, and at times, apparently at least, altogether wanting. The section represented in Figure 8 gives a good view of the relations of the various parts of the cell at this time; it passes approximately through the center of the starch mass and a little below the center of the nucleus. The starch grains are numerous and frequently aggregated in small groups, commonly, although not invariably, four in a group. The apparently finely granular material between the grains now tends to take a reddish to an orange stain. The nucleus still contains numerous nucleoles.

Usually it is difficult to trace the nuclear membrane in the region where the starch mass is in contact with it. This is true of the stages as early as those represented in Figures 5 and 6. The appearance of the preparation at times suggests that the nuclear membrane may be breaking down at that point;

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[15] Osterhout, W. J. V.: Spindle formation in *Agave*. *Proc. Cal. Acad. Sci.*, 3rd ser., Bot., ii (1902): 262, Pl. XXVI, Figs. 9–11, also Pl. XXV.

it seems probable, however, that the difficulty in distinguishing the nuclear membrane here is due to the over- and underlying dense starch mass; at any rate the starch mass will give such an appearance as is found even if the nuclear membrane is of uniform thickness throughout.

The fact that the spore mother-cells of *Marsilia* show a distinct polar organization from an early period in their development, makes it seem likely that the forming spindle should be bipolar from the beginning of its appearance, an expectation which is increased by the early appearance of a bipolar arrangement of the fiber groups in the cytoplasm. A study of the spore mother-cells at the time when the nuclear membrane has just disappeared shows, however, that apparently this expectation is not realized. The spindle fibers, which by that time have assumed the smooth, clean-cut appearance characteristic of the fibers as found in the completed spindle, are not arranged in a bipolar figure, but in several perfectly distinct and separate poles. The spore mother-cells of *Marsilia* show as striking "multipolar" spindles as are found anywhere. Figures 9*a* and 9*b* represent two consecutive sections of a spore mother-cell with four well-developed poles; 9*a* lies above 9*b*, and the starch mass lies in the section above that from which 9*a* is taken. Fibers from each of the four poles run up to the chromosomes, and in addition there are some running more or less continuously from one pole to another; this is to be seen in the two lower poles of the cell figured. There are also some fibers connecting the other poles, but they are not so numerous nor so conspicuous. The idea suggests itself, but it is only a suggestion, that the two poles conspicuously connected by fibers will be the definitive spindle poles. One of the four poles lies in close contact with the plasma membrane, two others have at least some fibers running up to the plasma membrane, and the fourth one lies distinctly free from it, although it comes close to it. That this pole is not in contact with the plasma membrane in a plane above or below that of the drawing can be easily determined by focusing up and down.

It is a fact worth noting that many of the "multipolar" spindles which have been figured are quadri- and tripolar

spindles (the latter possibly also, in many cases at least, really quadripolar); furthermore, as has frequently been pointed out, multipolar spindles have been described so far practically only for spore mother-cells. To mention a few instances, Berghs figures a roughly tripolar spindle for *Narthecium ossifragum,*[16] Grégoire and Berghs figure several for *Pellia epiphylla,*[17] and Miyake several for *Galtonia candicans,*[18] and one for *Tradescantia virginica.*[19] It would be interesting to know in how many cases the spindle of the first division in spore mother-cells starts as a quadripolar figure. It seems that good multipolar stages are difficult to find in most plants.[20] Frequently cells figured for such stages show evidence of poor fixation. It is a common experience that the multipolar stage is difficult to fix well. All this merely emphasizes the necessity of caution in determining whether there is in general any indication that the spindle for the first division in spore mother-cells starts as a quadripolar figure.

Unfortunately, the stages intervening between the disappearance of the nuclear membrane and the establishment of the bipolar spindle appear to be passed over rapidly in *Marsilia,* so that they are only occasionally found in the fixed material, and then it seems that they are difficult to fix well; at any rate, I have found very few satisfactory preparations of this stage. Strasburger does not figure any stages between diakinesis with the nuclear membrane intact and the practically completed bipolar spindle; he mentions a multipolar stage for *M. quadrifolia,*[21] but the section represented in his figure shows only two poles. In his Figure 86 Strasburger represents a spore mother-cell of *M. Drummondii* in diakinesis with two well-developed spindle poles. It is perhaps a question, however, whether these represent the definitive spindle poles.

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[16] *La Cellule,* xxii, Pl. I, Fig. 23.

[17] *La Cellule,* xxi, Pl. II, Figs. 1 and 2.

[18] *Jahr. f. wiss. Bot.,* xlii, Pl. III.

[19] Ibid., Pl. V.

[20] Mottier figures excellent ones for *Lilium* and *Podophyllum* (*Jahr. f. wiss. Bot.,* xxx, Pls. III and IV, and xxxi, Pl. II, Fig. 4), and Osterhout for *Equisetum* (*Jahr. f. wiss. Bot.,* xxx, Pl. I).

[21] L. c., p. 146; Fig. 60.

The chromosomes at the stage represented by Figure 9 are crowded near the central part of the cell, a condition which suggests a pushing in on them on the part of the spindle fibers. In this connection it should also be mentioned that at one stage shortly after synapsis, when the chromatin strands are long and of fairly uniform diameter, they apparently lie in a loose tangle on the side of the nucleus opposite that at which the synaptic mass was located (Figs. 5 and 6). It looks as though the chromatin strands were pushed over to that side, although the cytoplasmic fibers cannot be traced at this stage into the nuclear cavity.

As the nuclear membrane disappears during diakinesis and the spindle for the first division forms, the starch mass apparently continues to occupy the same position in the cell that it had during the earlier stages. In presynapsis and early synapsis stages, the starch mass invariably lies on the outer side of the cell; later it is frequently found on the side nearest the interior of the spore sac or in some intermediate position. It is to be noted, however, that at just about this time the spore mother-cells commence to round off and separate more or less from each other, and it seems that at this time they rotate more or less so that their original position is changed. At another time I hope to enter more into detail upon the motions which the spore mother-cells undergo at this period; suffice it for our present purposes to say that, as far as my data go at present, they do not indicate that the starch mass undergoes any translocations within the cell up to the time of the first nuclear division.

The spindle figure for the first division is unusually sharply defined. The fibers are delicate, numerous, and take a brilliant blue with the triple stain; they are sharply differentiated, so that they are readily followed in cross sections of the spindle. The poles are often rather broad and lie close to the plasma membrane, although they do not necessarily extend to it. The fibers twist about each other and frequently come together in little groups at their polar ends, and often a small, deeply staining granule lies at the end of such a group. There seems to be no constancy in the number of such groups in a spindle.

Distinct fibers running continuously from pole to pole can be easily distinguished between others, often considerably heavier, which end at the chromosomes (Figs. 10 and 11). In the metaphases, the spindle at times shows a peculiar zonated appearance, a dark band running across it in one or two places between the equatorial plate and the poles. These dark bands are symmetrically placed in the two halves of the spindle and seem to be due to a thickening of the fibers at these regions. This suggests the possibility that in the living cell there may be waves of contraction passing over the spindle. The fact that such zonated spindles are not often found in fixed material does not argue strongly against this possibility, for it is only under an exceptionally favorable combination of circumstances that the fixation of such "waves" could be expected. A more important argument against this possibility lies in the fact that in studies which have been made on karyokinetic figures in living cells nothing has been observed, as far as I am aware, of waves of contraction passing over the spindle. The difficulty of observation here, however, discounts the conclusiveness of a negative result.

The chromosomes on any spindle show considerable differences between themselves both in form and size, differences which are, if anything, even more marked in the late diakinesis stages and shortly after the breaking down of the nuclear membrane. Equally remarkable is what may be called a physiological difference between the chromosomes. There are regularly found around the periphery of the spindle small chromosomes whose halves separate considerably earlier than those of the other chromosomes, so that they are halfway to the poles when the halves of the others are still together at the equator of the spindle. It seems that for my specimens of *M. quadrifolia* this is a regular occurrence. Strasburger observed that in the equatorial plate stages of *M. drummondii* a varying number of chromosome pairs separate before the others; but he finds this associated with a more or less abnormal course of division, and refers it to a diminished attraction between homologous chromosomes. In its extreme case, this diminution in

the attraction between homologous chromosomes gives rise to diploid figures.[22]

It is to be noted that the axis of the spindle figure lies at right angles to the original main axis of the cell. The starch lies as a more or less flattened mass of oval outline to one side of the spindle. Rarely do any of the starch grains lie among the spindle fibers, or spindle fibers extend into the region of the starch mass. As the chromosomes collect at the poles and the daughter nuclei are reconstructed, a conspicuous system of connecting fibers develops between them. The fibers are heaviest at the middle and become more delicate and interlaced as they approach the nuclei. In addition to these connecting fibers, there is a more or less well developed system of polar radiations extending out from the region of the nuclei; as a rule these radiations are not conspicuous in the first division.

During, or somewhat previous to, the telophases, definite and characteristic changes occur in the position of the starch mass. It gradually moves in between the two daughter nuclei. In so doing it passes around the periphery of the spindle faster than through the spindle itself, and as a result the starch mass is for a time roughly horse-shoe-shaped. In the cell represented in Figure 12, the starch extends almost completely across the cell in the planes above and below the spindle, but in the plane of the spindle itself it is not halfway across. As the starch mass is coming in between the spindle fibers, the latter are disappearing. As is well known, cell division does not follow the first nuclear division in the spore mother-cells of *Marsilia*. The connecting fibers, however, frequently persist for a considerable period, and it is not uncommon to be able to trace them between the starch grains. The starch mass finally forms a relatively thin plate lying midway between the two daughter nuclei and extending almost to the periphery of the cell. It is to be noted that its plane is now at right angles to the position it occupied at the time of the equatorial plate stage and earlier.

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[22]L. c., pp. 152, 155; Figs. 90, 102.

The chromatin of the reconstructed daughter nuclei is often largely confined to the periphery of the nucleus, and nucleoles reappear. In the prophases of the second division there is a conspicuous development of fibers, and in this division it is not difficult to find pronounced multipolar stages in the formation of the spindle. The fibers are again characterized by an unusually clean-cut appearance; they envelop the chromosomes in a dense tangle, and at their distal ends they come together in numerous places to form sharply pointed poles, each of which consists of relatively few fibers (Fig. 13). Frequently the poles are somewhat curved. The upper spindle of Figure 13 is cut to one side, most of the chromosomes lying in the next section. The completed spindle again is a well defined figure. It is more slender than that of the first division, and the poles tend to be somewhat sharper, but the individual fibers stand out with the same distinctness and take the same brilliant stain as in the first division.

The orientation of the spindles of the second division shows some interesting variations; as is frequently the case in the second division in spore mother-cells, the two spindles lie at various angles to each other. In some cases the two spindles and the starch mass lie in three parallel planes. The axes of the spindles may then be parallel to each other, at right angles to each other, or at some intermediate angle. In other cases, however, the plane of one or both of the spindles is at an angle to that of the starch mass, and then it frequently happens that the plate-shaped starch mass is somewhat deformed. Figures 14*a* and 14*b* represent two successive sections of such a cell; 14*a* lies above 14*b*. The lower spindle lies in the plane of the section, and ordinarily the starch mass would lie some distance above it, also in the plane of the section. In this case, however, the upper spindle is at an angle to the plane of the section, the right hand pole lying lower, the left hand one higher, and the starch plate has been depressed on the right hand side of the cell. The starch plate apparently continues to form a rather complete separation between the spindle-figures of the second division until late in the anaphases of that division.

As the chromosomes move back to the poles, the polar ends of the spindle become markedly darker and more compact (Fig. 14*b*). This stands out even more strikingly in the preparations than in the drawing, where only a section of the spindle is represented. The spindle shown in Figure 14*a* is essentially of the same appearance as that in Figure 14*b;* the right hand pole, however, is represented lighter because it lies deeper in the section; the left hand (higher) pole, on the other hand, has a large proportion of its fibers in the next section above, so that it does not appear heavy in the drawing. The spindle figures at this stage show well-developed connecting fibers running from pole to pole, and it can readily be seen that some of them lie distinctly outside of the chromosome group. Delicate radiations extend out from the poles of the spindle towards the equatorial region; at times radiations from the two poles cross in the equatorial region. Although delicate, these fibers because of their distinct blue stain stand out sharply in the preparations against the orange-gray background of the other cytoplasmic structures. The poles of the spindles frequently do not reach to the plasma membrane. Occasionally, in what otherwise seem to be somewhat abnormal cells, the spindle poles are unusually sharp and compact and end directly at the plasma membrane; if the cells are somewhat shrunken, the plasma membrane is markedly pushed out at the points where the spindle poles are in contact with it. More or less curved spindles also are not rare.

After the chromosomes reach the poles and the daughter nuclei are reconstructed, the starch is so distributed that about an equal amount is contained in each of the four cells resulting from the development of cell-plates by means of the connecting fibers between the nuclei.

The spore mother-cells of *Marsilia* show for a considerable period of their existence a well-marked polar organization. In the earlier stages, the visible expression of this as far as the cytoplasm is concerned is the characteristic position of the starch grains in the cell. There is no evidence of their being enclosed by an enveloping membrane as is the case in young

leaf cells of *Isoetes.*[23] Later, during the processes of nuclear division in the spore mother-cells of *Marsilia,* as has been described, the starch grains undergo definite and specific translocations; but these translocations are conspicuously different from those of the starch grains in young vegetative cells of *Isoetes.* This difference is at least partly due to the fact that in these cells of *Isoetes* the starch grains are confined to the interior of the "polar structure," and hence necessarily change their position as its position changes. The cells of *Marsilia* apparently do not possess a structure like the "polar structure" of *Isoetes.* It is a striking fact that in *Marsilia* the often numerous starch grains, although as far as apparent not enclosed in any kind of an enveloping membrane, remain fairly closely packed in a single group from soon after the time of their first appearance in the cell until the completion of the second division; and we have in the specific changes of position and form which this group of starch grains undergoes an interesting point of attack upon the problem of the forces operating in the cell during nuclear division. Starch grains, however, do not universally show in their position and movements a marked effect of the forces operating in the cell during nuclear division. The figures of Grégoire and Berghs for the early divisions in the germinating spore and in the spore mother-cells of *Pellia epiphylla*[24] are of interest in this connection. There the starch grains are apparently scattered quite irregularly throughout the cytoplasm (not, however, through the karyokinetic figure).

Contrary to what might be expected, the polar organization of the spore mother-cells of *Marsilia,* which is so conspicuous during the earlier stages, seems to have quite disappeared at about the time when the nuclear membrane breaks down, or at any rate it seems to find no expression in the arrangement of the spindle fibers just preceding the formation of the bipolar spin-

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[23] Marquette, W.: Manifestations of polarity in plant cells which apparently are without centrosomes. (*Beih. bot. Centralbl.*, xxi : 281. 1907).

[24] Grégoire, V., and Berghs, J.: La figure achromatique dans le *Pellia epiphylla. La Cellule,* xxi :193. 1904.

dle. It will be necessary to have more extensive data on the orientation and position of the fiber groups at this stage before the relation of the "multipolar" (or quadripolar) spindle to the earlier organization of the cell can be advantageously discussed.

It is as yet impossible to judge whether in general a multipolar origin of the spindle is to be regarded as inconsistent with a permanent polar organization of a cell. Careful study may show that even during the multipolar stages, where they occur, the original polar organization of the cell is maintained, and that the cell-axes can be traced continuously.

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EXPLANATION OF PLATE VIII.

The figures of this and the following plate were drawn with the aid of the camera lucida, the magnification about 1,700 diameters in each case. All figures are from spore mother-cells of *Marsilia quadrifolia.*

Fig. 1. Young spore mother-cell, showing characteristic aggregation of chromatin at central part of nucleus.

Fig. 2. Somewhat older spore mother-cell, showing an elongated, vacuolar region in the cytoplasm at *a.*

Fig. 3. An approximately full-sized cell; aggregation of starch grains on the side of the nucleus facing the sporange wall.

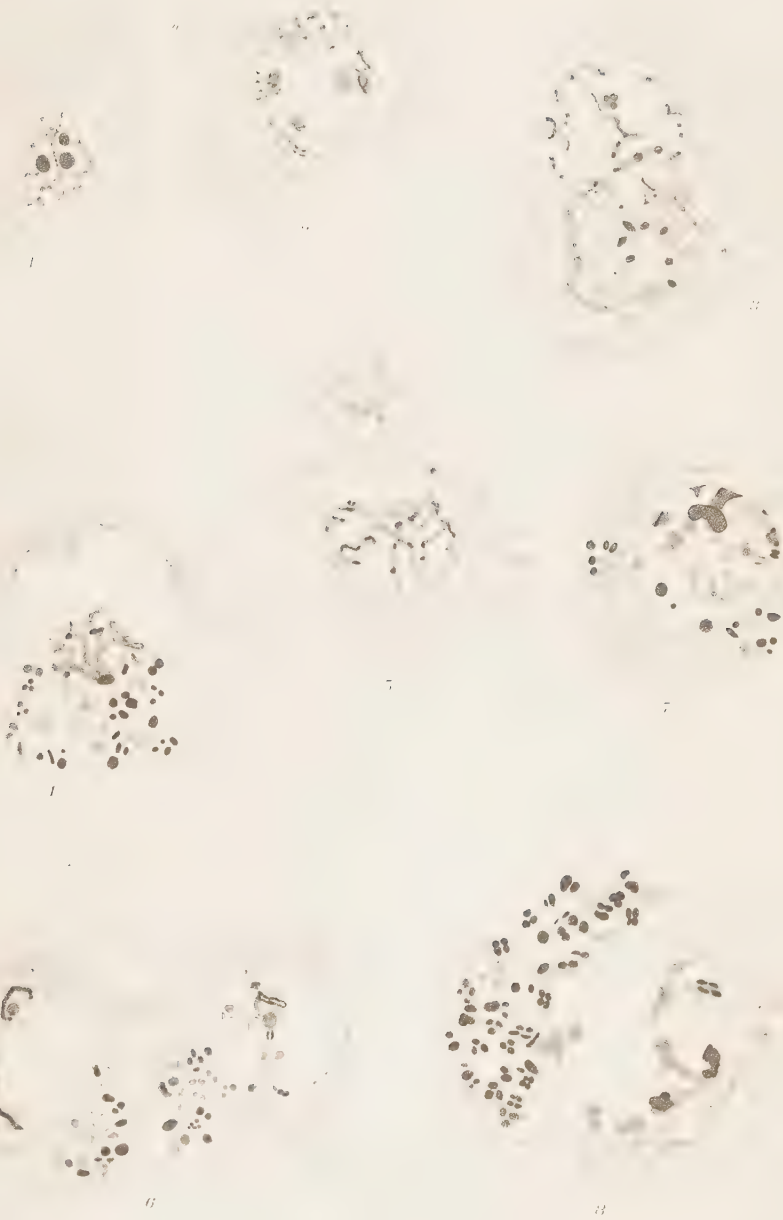
Fig. 4. Synapsis; the chromatin strands oriented on a nucleole lying close to the nuclear membrane.

Fig. 5. Post-synapsis: the chromatin strands lying on the side of the nucleus opposite that occupied during synapsis. A fairly well-developed bipolar figure of fibers in the cytoplasm, an indication of a third pole at the lower left corner. Most of the starch mass is in the next section.

Fig. 6. Two cells in approximately the same stage as the preceding. In one of them a granule simulating a "centrosome."

Fig. 7. A later stage, showing unsymmetrical arrangement of the cytoplasmic fibers; a number of cone-shaped groups lie at the lower left corner of the cell below those shown in the figure. A considerable number of starch grains lie above the plane of the drawing.

Fig. 8. Diakinesis.



W. Marquette del.

PLATE IX.

EXPLANATION OF PLATE IX.

Figs. 9 *a*, *b*. Two successive sections of a cell showing a quadripolar spindle. 9*a* lies above 9*b*, and the starch mass is in the section above 9*a*.

Figs. 10 and 11. Completed spindle. Some chromosomes are well on the way to the poles, while others are still at the equator of the spindle.

Fig. 12. Reconstructed daughter nuclei. The starch mass moving in between the nuclei; in the planes above and below that of the drawing it reaches almost completely across the cell.

Fig. 13. Multipolar stage in the formation of the spindles in the second division. The starch mass forms a plate across the cell separating the two figures.

Figs. 14 *a*, *b*. Two successive sections of cell in anaphases of second division, 14*a* above 14*b*. The spindle represented in 14*a* lies at an angle to the plane of the section, the left hand pole being higher (part of it lies in next section above), the right hand pole lower and hence represented lighter. The left pole is above the starch plate. The starch plate is depressed by the right pole and appears in part in Figure 14*b*. The spindle shown in 14*b* lies in the plane of the section. The spindle poles of 14*a* are as well developed as those of 14*b*.

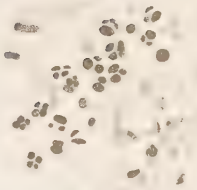
9a

9b

10

11

12



13

14a

14b

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